

The effect of lactoferrins on ETEC growth and attachment to intestinal epithelial cells

Matthias Dierick, Bert Devriendt, Daisy Vanrompay, Eric Cox

Laboratory of Immunology, Faculty of Veterinary Medicine, Ugent

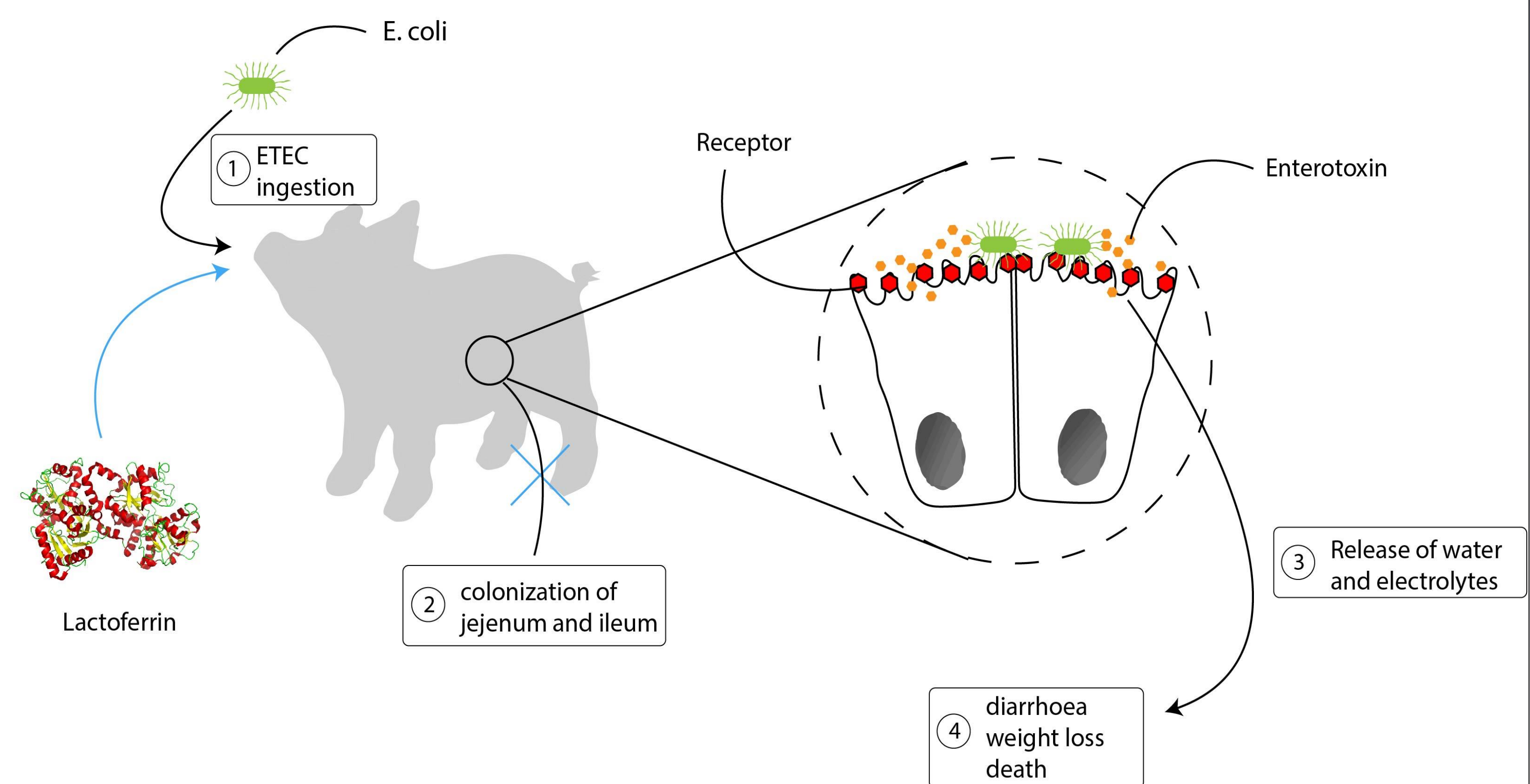
Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ugent



Background

Enterotoxigenic F4⁺ and F18⁺ *E. coli* (ETEC) infections are a major cause of intestinal infections in neonatal and recently weaned piglets, resulting in diarrhoea, growth retardation, mortality and elevated use of medications. Extensive use of antibiotics and zinc oxide during the first two weeks after weaning is used to control post-weaning diarrhoea and has most likely contributed to an increased occurrence of antibiotic resistant strains.

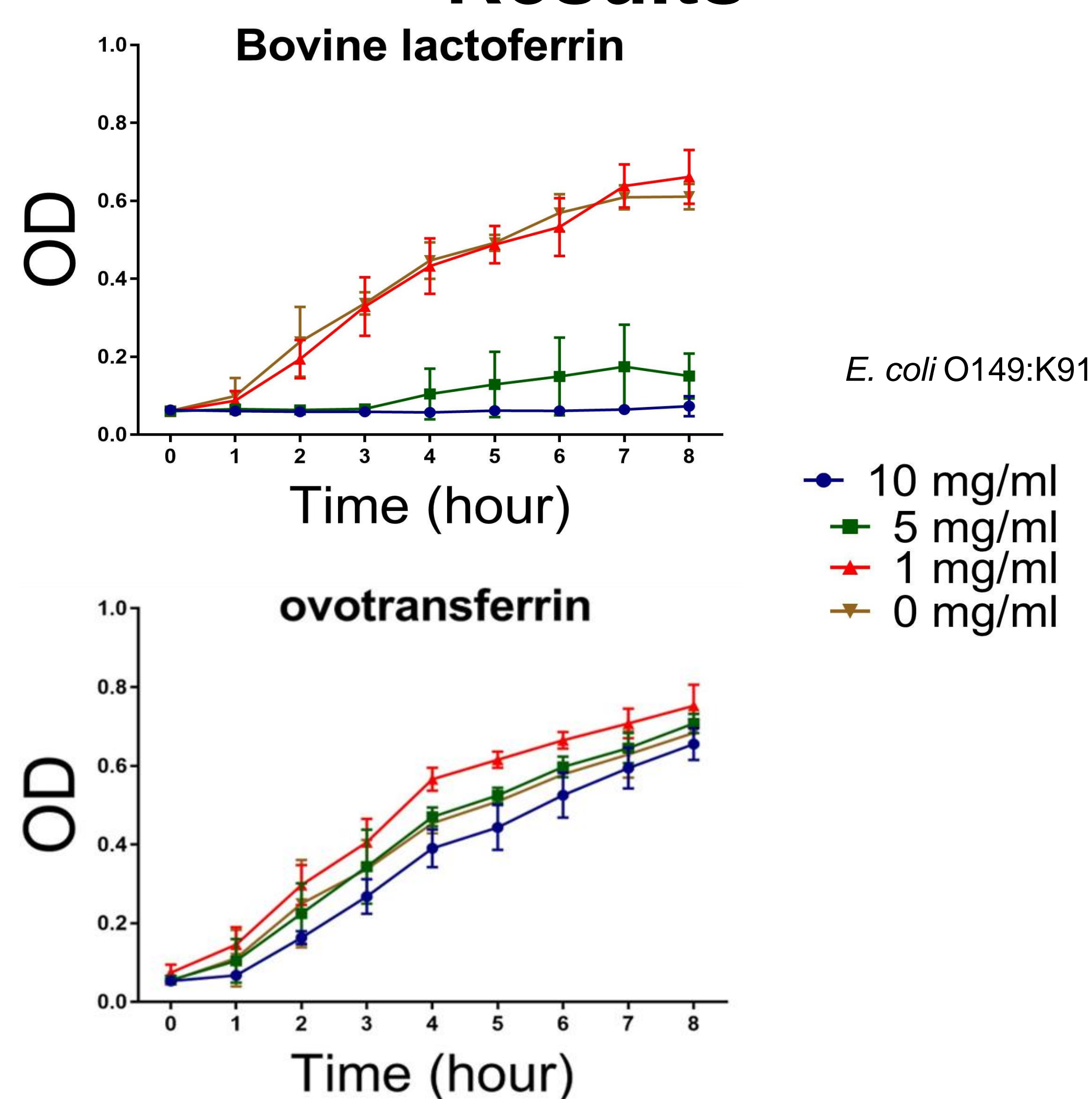
A possible strategy to overcome this problem is to use naturally derived molecules, which can decrease infection by a direct effect on the microorganisms and/or increase host resistance, have recently gained interest. One of those molecules is lactoferrin. Lactoferrin is a naturally derived molecule, which has been shown to have antibacterial activity by a direct effect on the microorganisms and/or increase host resistance.



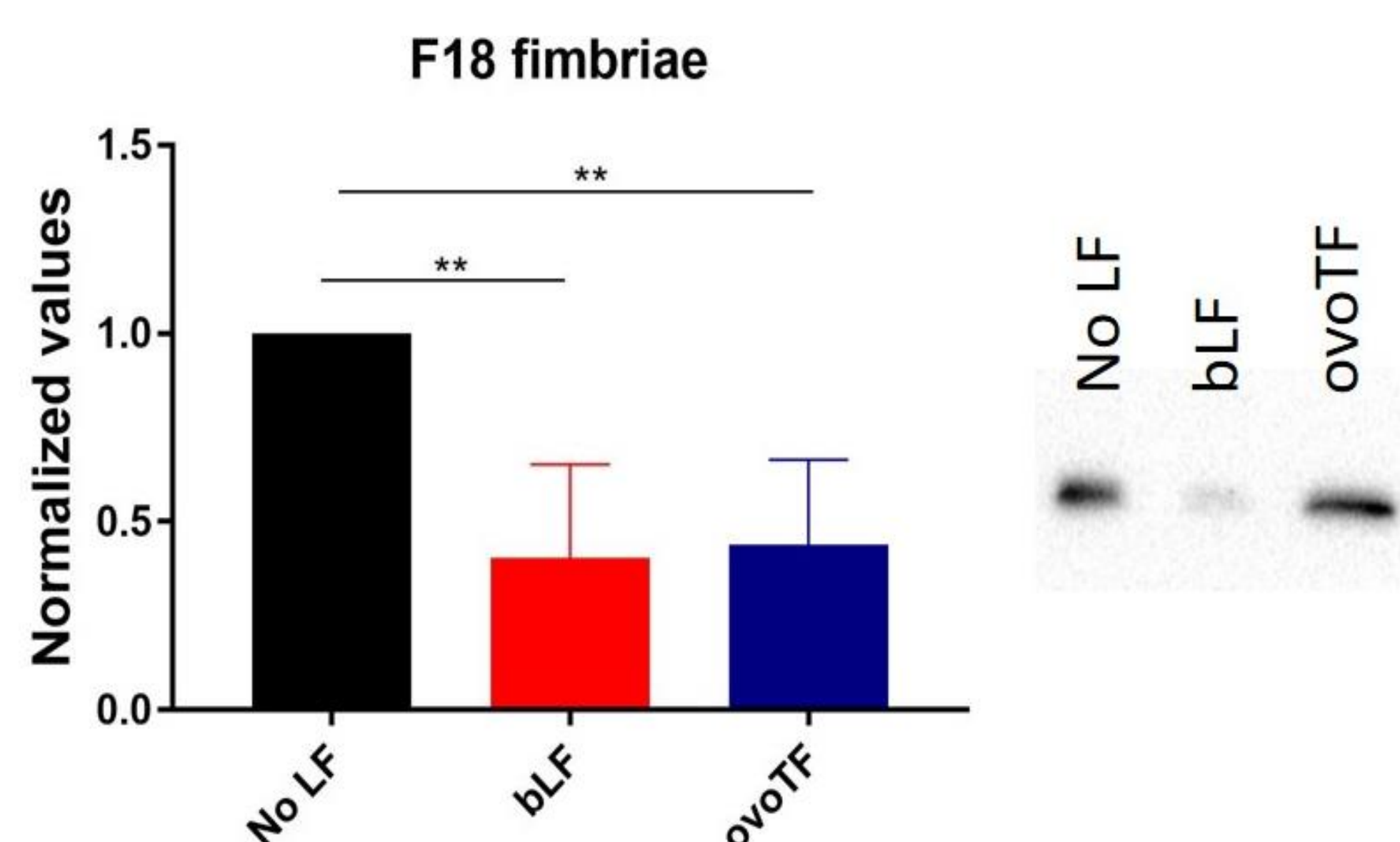
Aim

To evaluate the direct effect of lactoferrins from different species on bacterial growth, bacterial adhesion to enterocytes and stability of fimbriae, flagellin and enterotoxins.

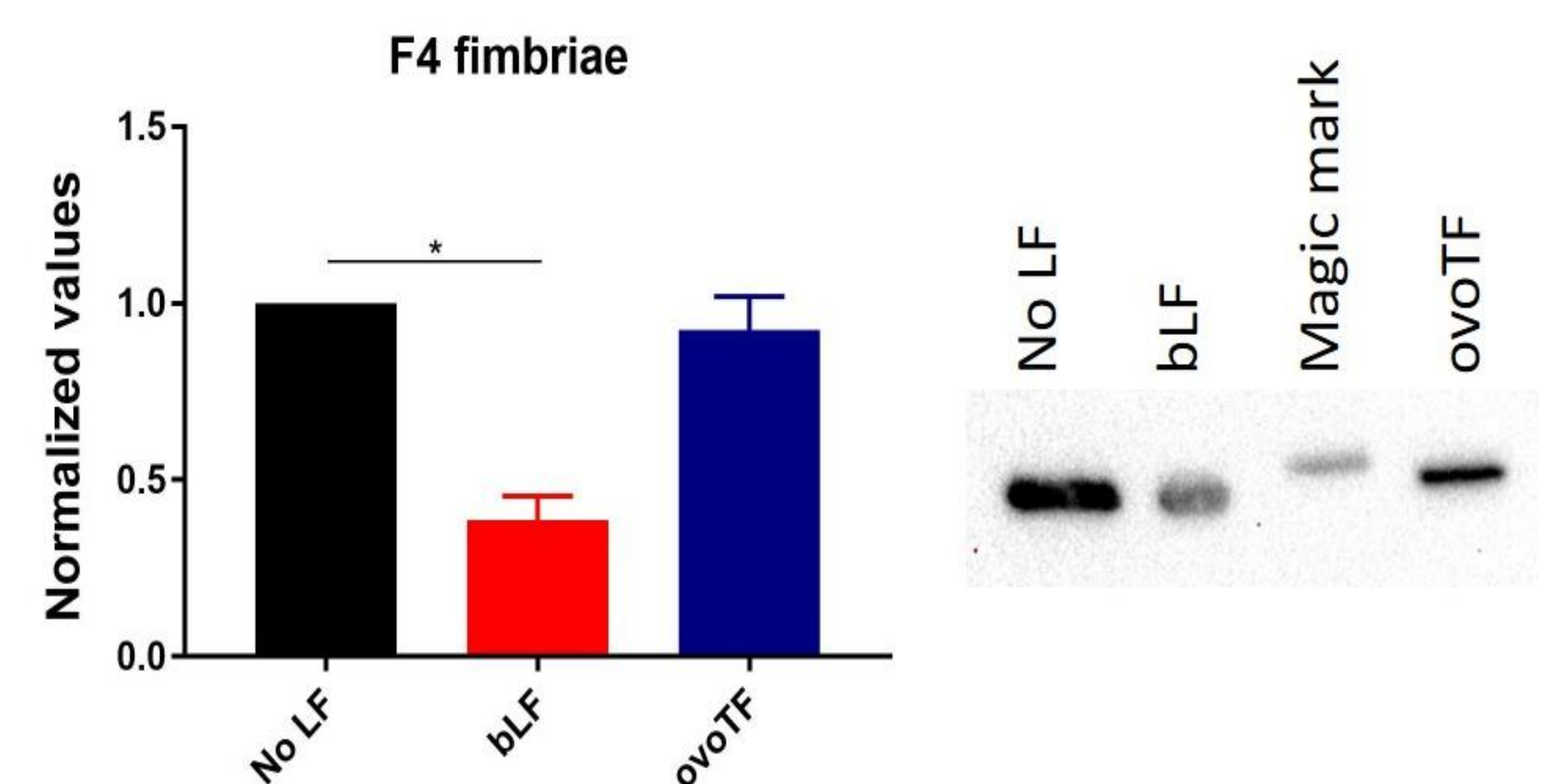
Results



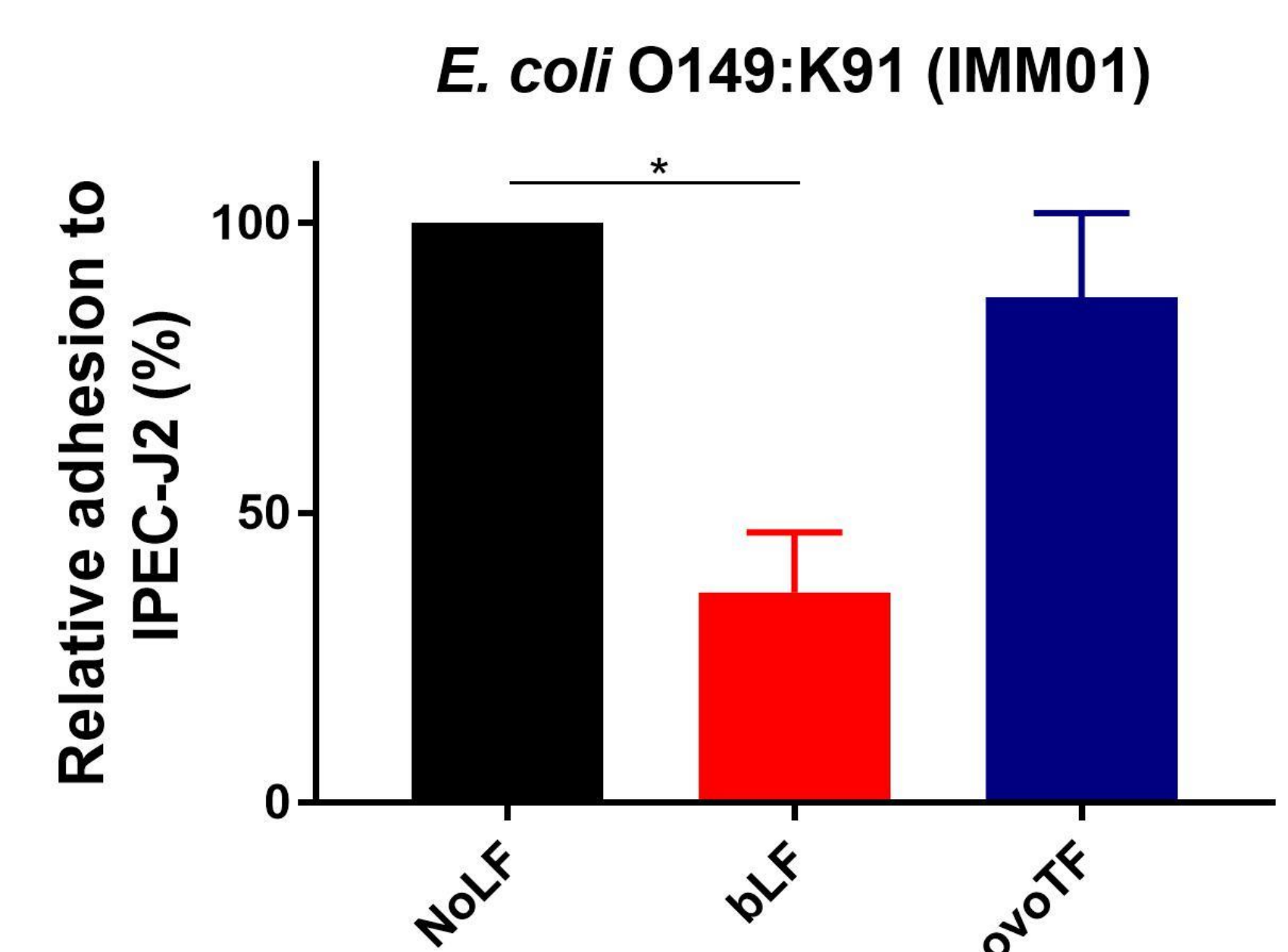
Bacteria were incubated in LB medium with different concentrations of bovine lactoferrin or ovotransferrin and the OD was measured every hour. The results are represented as the mean OD \pm S.E.M. ($n = 3$). Growth inhibition was observed with the two highest concentrations of bovine lactoferrin (10 and 5 mg/ml), but not with ovotransferrin.



Bovine lactoferrin (bLF) and ovotransferrin (ovoTF), which are known to have serine protease activity, were shown to degrade F18 fimbriae at a concentration of 10mg/ml. The relative quantification of F18 fimbriae degradation (left) with a representative western blot (right). Based on 7 independent measurements, Data are presented as the mean \pm sd.



Only bovine lactoferrin is capable of degrading F4 fimbriae. The relative quantification of F4 fimbriae degradation (left) with a representative western blot (right). Based on 4 independent measurements, Data are presented as the mean \pm sd.



In an adhesion assay, *E. coli* O149:K91 was incubated with IPEC-J2 cells in the absence or presence of bovine lactoferrin (bLF) and ovotransferrin (ovoTF). We showed that both bovine lactoferrin can decrease the attachment of F4 fimbriated *E. coli* O149:K91 to small intestinal epithelial cells.

Conclusions

Only bovine lactoferrin is capable of inhibiting bacterial growth and degrading F4 and F18 fimbriae. In an adhesion assay, we further showed that bovine lactoferrin decreases the attachment of *E. coli* O149:K91 to small intestinal epithelial cells.

An *in vivo* challenge experiment is planned to determine the effect of lactoferrin on the attachment of *E. coli* to the intestinal epithelial cells, excretion of *E. coli* and mucosal immune response